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The aberrant activation of the ErbB2 receptor tyrosine kinase activity appears to play an active role in the progression of some mammary tumors to malignancy. It is therefore of value to identify compounds that efficiently and specifically inhibit ErbB2 kinase activation. Using cyclic peptide library technology we have developed a method for screening up to 17 billion related compounds in a single experiment for the ability to bind with high affinity to the ErbB2 receptor extracellular domain. Libraries were designed based on the Loop3 structure of the EGF-like growth factors because preliminary observations indicated that a modified form of this subdomain could act as an antagonist of ligand-stimulated ErbB2 activity. Cyclic peptide libraries were synthesized containing ten to 15 residues, where up to eight positions were each 19-fold degenerate in the natural amino acids (excluding cysteine), and at least five positions were fixed with non-degenerate orienting amino acids. Libraries were then incubated at 100-fold molar excess with purified ErbB2 extracellular domain, so that those peptides that bind to ErbB2 with highest affinity were selected from the mixture. Receptor-bound peptides were then isolated and sequenced by Edman degradation to determine the optimal receptor binding motif provided by that library. While specific binding to the receptor extracellular domain was observed, these did not yield any significant high affinity binding motifs.

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Table of Contents

Introduction

The purpose of the studies outlined here was to develop a novel degenerate cyclic peptide library selection method to identify potential peptide antagonists of the ErbB2 receptor tyrosine kinase. Since the aberrant activation of the ErbB2 receptor is believed to be involved in the genesis or progression of a significant proportion of human breast tumors, a cyclic peptide antagonist that binds selectively to the extracellular domain of ErbB2 could eventually be useful as an anti-breast cancer therapy. To identify potential antagonists, the extracellular ligand binding domain of the ErbB2 was used to affinity purify cyclic peptides from oriented random peptide libraries. The structures of oriented peptide libraries were based on the Loop3 region of the dozen known peptide ligands specific for other members of the ErbB receptor family. Amino acid residues conserved in the known ligands were preserved, while those that vary were made degenerate at their corresponding positions in the libraries to select for high affinity binding to ErbB2. We anticipated that a high affinity synthetic peptide ligand for ErbB2 would be identified by this approach, which was then to be assessed for its ability to act as an antagonist for the receptor. However, the results from two of the three tested libraries indicated that while binding of library peptides to the ErbB2 extracellular domain could be observed, a preferential selection of residues at degenerate positions of the library could not be observed.

Body

Because of its central role in the genesis and progression of a significant proportion of breast tumors, the ErbB2 (HER2/neu) receptor tyrosine kinase is a very attractive target for the development of anti-cancer agents (1,2). This is illustrated by the FDA approval of Herceptin, a humanized anti-ErbB2 antibody, for administration to patients that have tumors overexpressing this protein. While Herceptin does exhibit significant promise in the clinic, particularly when administered in combination with more tradition chemotherapies (3), the *in vitro* observation that Herceptin acts as an ErbB2 agonist rather than antagonist (4) raises issues as to potential side effects in ErbB2-expressing tissues. Indeed, it has been observed that a subset of patients treated with Herceptin develop cardiac abnormalities, which subside when the patients are taken off the drug (5). Moreover, Herceptin is administered to patients whose tumors overexpress the ErbB2 protein. While overexpression certainly leads to the constitutive activation of the kinase activity of this receptor, autocrine ErbB2 stimulation mechanisms are also quite common in many solid tumors (6). These observations demonstrate that ErbB2 is a viable target in the battle against aggressive breast tumors, but raise the question as to whether a more reliable ErbB2-targeted drug could be developed. The role of an ErbB2-selective antagonist in patient therapy remains to be tested.

The purpose of the studies outlined in this report was to develop a screening method for the identification of high affinity antagonists of the ErbB2 receptor by exploiting information currently available on the mechanism of its activation by EGF-like growth factor ligands. In tumors ErbB2 may be constitutively activated by the autocrine expression of at least one of more that a dozen growth factors. No known diffusible ligand binds directly to ErbB2 to stimulate its activity, but it appears that ErbB2 is a co-receptor and the central target of activation of all of the EGF-like growth factors (7,8; see Figure 1). During development the co-activation of ErbB2 along with the primary growth factor receptors EGF receptor, ErbB3 or ErbB4, is thought to augment or modify the cellular response to the stimulus. In tumors, ErbB2 activation is thought to contribute to progression. Hence a key question concerns the mechanism by which EGF-like ligands activate ErbB2.

The most widely accepted model for ErbB2 activation states that EGF-like ligands (illustrated in Figure 2) are actually bivalent and make contact with both the primary binding ErbB receptor (EGF receptor, ErbB3 or ErbB4) and the heterodimerizing receptor (ErbB2). Figure 3 shows the bivalency model developed by Tzahar et al. (9). Their studies suggested that high affinity, narrow specificity binding to the primary receptor is conferred by amino-terminal sequences of EGF-like growth factors, while lower affinity, broader specificity binding to the heterodimerizing receptor is conferred by carboxy-terminal sequences. Their previous work had shown that despite the broader specificity conferred by the carboxy terminus there is still a significant degree of selectivity. We reasoned that since this region (the Loop3 region of the EGF-like ligands, defined by their fifth and sixth cysteine residues as well as immediately adjacent carboxy-terminal sequences) was responsible for making contact with ErbB2 it would provide a suitable basis on which to build binding antagonistic peptides. Indeed, our preliminary data using a slightly modified form of the Loop3 region of the EGF-like growth factor Neuregulin-1β (NRG1β) indicated that this cyclic peptide could inhibit the ErbB2 tyrosine phosphorylation stimulated by intact NRG1β in MCF7 cultured breast tumor cells with a K_i of 1 μM (K.L. Carraway III, unpublished observations). The purpose of the proposed studies was to use these observations as a starting point to develop higher affinity, higher selectivity Loop3-based cyclic peptides that might act as antagonists for ErbB2.

The strategy used for these studies is illustrated in Figure 4. Three Loop3-based cyclic peptide libraries were generated and independently mixed with purified recombinant ErbB2 extracellular domain (2ECD) in a 100-fold excess of peptide to protein. 2ECD was allowed to select high affinity binders, excess peptides were removed, and bound material was eluted and sequenced by Edman degradation. The relative content of each amino acid at each degenerate position was then compared with the original library to determine the optimal motif for ErbB2 binding.

Specific Aim I: Express the extracellular domain of ErbB2 in milligram quantities and purify the expressed product to homogeneity.

We originally expressed the ErbB2 extracellular domain in High Five insect cells as a glutathione-S-transferase (GST) fusion protein using baculovirus technology. The cDNA encoding human ErbB2 extracellular domain was amplified by polymerase chain reaction, subcloned into the insect cell transfer vector pAcSecG2T (Invitrogen), and confirmed by sequencing. The resulting construct contained an insect cell-specific leader sequence followed by the GST moiety, fused in frame with residues 24-652 of human ErbB2. This material was used to generate recombinant baculovirus according to our standard protocols (10,11). Recombinant viruses were plaque purified, amplified and used to infect High Five insect cells (Invitrogen) to produce the recombinant protein. High Five cells were grown in ExCell 400 media (J&H Bioscience) in the absence of serum, minimizing contamination from other proteins. Media was supplemented with 0.1 mg/ml BSA as a carrier that could be easily purified away. This fusion protein was recognized by five out of five tested conformationally-sensitive antibodies to the ErbB2 extracellular domain (Year 1 report), strongly suggesting that the expressed protein is functionally similar to that found in breast tumor cells.

However we found that the GST fusion protein was difficult to purify in high yield because of a weak interaction with immobilized glutathione. Typically we could recover only 10% of the expressed material upon purification with reduced glutathione. The reason for this is unknown but may reflect either steric hinderance of GST binding to beads by 2ECD or the oxidation of the GST moiety after secretion but prior to purification. Rather than repetitively purifying the expressed material to generate high quantities, we re-engineered the ErbB2-ECD construct to contain a 6X-His tag at its amino terminus. With this construct we replaced the leader sequence (the first 23 amino acids) of human ErbB2 (12) with the leader sequence of a baculoviral glycoprotein to optimize expression and secretion in insect cells. This was carried out using a commercially available plasmid (pAc67B, Invitrogen) that adds this leader to a subcloned sequence. This leader was followed by six histidine residues and three glycine residues, and then attached to the ErbB2 extracellular domain at residue 24. We engineered a stop codon at residue 653 immediately N-terminal to the start of the transmembrane domain. Again, purified recombinant baculovirus was generated and used to infect Hi5 insect cells using our standard expression protocols, generating typical yields of 1 mg/2.5x10^8 cells. Figure 5A shows the purification of His-2ECD by affinity for nickel and elution with an imidazole gradient, while Figure 5B shows a Coomassie blue stain of the purified material. On the basis of this data we estimate that our purified material is at least 90% homogeneous. Figure 5C shows that His-2ECD may be immunoprecipitated by six anti-ErbB2 antibodies that recognize the extracellular domain in a conformation-dependent manner. Again, these observations strongly suggest that the purified material is functionally intact.

Specific Aim II: Develop an oriented peptide library approach to determine peptide motifs selected by the ErbB2-ECD.

On the basis of the Loop3 region of EGF-like growth factors, three cyclic peptide libraries were constructed (Figure 4). Our first synthesized library (library A) had the structure CXXXFXGXRC, where X represents a mixture of all natural amino acids excluding cysteine. Hence residues 2,3,4,6 and 8 are 19-fold degenerate yielding 2.5 million different compounds in the final library mixture. The advantage of this library is that it is entirely cyclic, and hence is predicted to be more stable *in vivo* than linear counterparts. However, Tzahar et al. (9) have suggested that sequences C-terminal to the Loop3 region are important in conferring affinity and specificity in mediating growth factor interaction with the dimerizing receptor. We therefore constructed the same library but added three degenerate residues to the C-terminus in an effort to enhance affinity. This library (library B) with the sequence CXXXFXGXRCXXX is 17 billion fold degenerate and has a lariat structure after cyclization. Finally, to further enhance affinity for ErbB2 ECD we added two more degenerate residues to the carboxy terminus of the library. However, if all of the residues within the loop were also allowed to vary the degeneracy of this library would be ridiculous and no specificity would be obtained. To circumvent this we kept the residues within the loop constant. These residues are identical to those in the beta isoform of neuregulin-1, a growth factor that potently stimulates the heterodimerization of ErbB2 with its primary binding receptors ErbB3 and ErbB4 (13,14). This library (library C) has the structure CPNEFTGDRCXXXXX, and is also 2.5 million fold degenerate.

We attempted to oxidize these libraries under a variety of conditions and found that the protocol described by Barbacci et al. (15) worked best. Efficient cyclization was achieved at low peptide concentration with minimal formation of library aggregates. Non-cyclized material was removed using immobilized N-ethylmaleimide, which covalently reacts with free sulfhydryl groups. From mass spectrometry (Year 1 report), we estimated that almost 70% of the library A material was monomeric cyclized peptide library, and we obtained 19 milligrams of the material. Similar results were obtained with libraries B and C.

Initial peptide library selection experiments were carried out using cyclized libraries A and B in the format illustrated in Figure 4, that is, association of peptides with His-2ECD immobilized on beads. In these experiments 1 mg (10 nmol) of purified 2ECD was covalently attached to 1 ml (packed) Affi-gel P10 beads (Bio-Rad), a commercially available cyanogen bromide-activated Sepharose that reacts with proteins through lysine residues. Non-reacted groups were then blocked with the free amine glycine. At least 90% of the His-2ECD was covalently bound to the support. In parallel beads were generated that did not contain His-2ECD but were blocked with glycine for use as a control in peptide binding experiments. Libraries A and B were dissolved in DMSO at 55 mg/ml and 70 mg/ml, respectively, and independently diluted 100-fold into 2 mls 1:1 slurry of His-2ECD or control beads, yielding a 100 fold molar ratio of peptide to His-2ECD. Binding was allowed to proceed 10 minutes at room temperature and then 2 hours at 4°C. Beads were then poured into 10 ml disposable columns and rapidly washed (~10 seconds) with 25 mls ice-cold PBS using vacuum to maximize the flow rate. [These washing conditions had been pre-established by testing the recovery of peptide after binding to control beads. Assuming a 1:1 stoichiometry of bound peptide to His-2ECD the most amount of specifically bound peptide to which we are entitled in our experiments is 10 nmol. Assuming a 90% loss of bound peptide during washing the most amount of recovered peptide to we are entitled to recover is 1 nmol. To achieve a 5-fold signal-to-noise ratio then, the highest amount of peptide eluted from control beads that can be tolerated is 200 pmol, or 0.02% of the starting peptide. Column washing conditions were established with these parameters in mind.] 1 ml 30%

acetic acid was then added to columns, and bound peptides were eluted, reduced with 50 mM DTT and reiteratively dried under vacuum with five 1 ml water washes. Reduced eluted peptides as well as aliquots of the original libraries were then sequenced by Edman degradation. Table 1, Part A shows the amount (pmols) of recovered phenylalanine in the fifth cycle and glycine in the seventh for peptides eluted from His-2ECD and control columns. No binding of peptide to His-2ECD over background was observed.

There are numerous possible reasons that no specific binding was observed in the column format experiment. First, the affinity of the cyclic peptides for His-2ECD could be too low for peptides to survive the washing necessary to minimize background binding to beads. Although washing was very rapid, binding of peptides with dissociation constants of 1 μM or greater is immediately reversible and will not be trapped by this method. Another possibility is that there is a flaw in the experimental design, particularly with regard to the covalent attachment of His-2ECD. It is possible that lysine residues through which His-2ECD is coupled to the support are also necessary for interaction with the Loop3 peptide. Alternatively the bead itself could sterically hinder access of the peptide to the binding pocket on His-2ECD.

To circumvent these issues we re-designed our experiments to examine library binding to His-2ECD free in solution. In these experiments, DMSO-solubilized libraries were diluted 100-fold into 1 ml PBS alone or PBS containing 1 mg/ml His-2ECD. The peptide:protein ratio was 100. After incubation at 4°C for two hours, unbound peptide was removed using an ice-cold 3 ml Sephadex G50 spin column centrifuged 2 minutes at 3000xg. [Again, the size of the column, the spin time and g-force were pre-determined optimizing for signal-to-noise.] The fluid, containing His-2ECD and bound peptide was then treated with acetic acid to 30%, and this mixture was centrifuged through a 10,000 molecular weight cutoff dialysis filter. The 100 kDa His-2ECD was retained and acid-eluted peptides passed through the filter. Peptides were reduced with 50 mM DTT, washed and sequenced together with the original libraries. Table 1, Part B shows the recoveries of peptides from His-2ECD in the new format. In this case we observed very significant specific binding to His-2ECD with both libraries A and B. However, when the selectivity factors were determined for the different amino acids at each degenerate position, no strong selection was observed. Table 1C shows the data for cycle 6 of the two libraries as an illustration. The selection factors were obtained in the manner determined previously (16-18), by dividing the mole fraction of each amino acid in the selected peptides by the mole fraction of the corresponding amino acid in the original library. Previous experience indicates that reproducible selectivity factors of 1.6 or higher are significant (16-18). In cycle 6, some selectivity factors of 1.6 were observed with one of the libraries but not the other. (It should be noted that the reproducibly high Phe selection at cycle 6 is undoubtedly a consequence of sequencing bleed-over from the fixed Phe in the previous cycle). Since library B is identical to library A except with three additional degenerate residues at the carboxy terminus, the library B experiment could be considered a replicate of the library A experiment for residues within the loop. Cycles 2,3,4 and 8 showed similar results; very few of the amino acids exhibited significant selection factors in a given library, and those factors were not repeated in the other library experiment. Cycles 11 and 12 of library B did show some significant selection, but these data suffered from low numbers (significant signal is lost in later cycles of sequencing) and the fact that the experiment was only carried out once.

Specific Aim III: Test selected peptides for antagonistic properties toward ErbB2/Neu.

Since no high affinity antagonists were found these studies were not carried out.

Key Research Accomplishments

- Expressed milligram quantities of functional tagged ErbB2 extracellular domain and purified this material to >90% homogeneity.
- Synthesized three degenerate oriented peptide libraries based on the Loop3 region of EGF-like growth factors.
- Efficiently oxidized libraries to form largely monomeric cyclic libraries to be used in screening for high affinity ErbB2 binding peptides.
- Screened two of the libraries against ErbB2 extracellular domain using two different experimental protocols.

Reportable Outcomes/Bibliography

Sweeney, C., Diamonti, A.J., and Carraway, K.L., III. A Novel Screen for Suppressors of Breast Tumor Cell Growth Using an Oriented Random Peptide Library Method to Identify Inhibitors of the ErbB2 Tyrosine Kinase. Era of Hope Meeting, June, 2000, Atlanta, GA.

Personnel

Diamonti, A.J.	Research Associate II
Carraway, K.L., III	Instructor in Medicine, Principle Investigator

Conclusions

The purpose of this study was to use cyclic peptide library technology to improve the binding of peptides to ErbB2 extracellular domain in an attempt to identify a high affinity antagonist. Our previous unpublished work had demonstrated that a form of the Loop3 region of the EGF-like domain of the growth factor NRG1 inhibited the stimulation of ErbB2 activity in MCF7 mammary tumor cells by intact NRG1 with a K_i of 1 μM. The driving hypothesis of this study was that this affinity could be improved upon by allowing the ErbB2 extracellular domain to choose the residues in the Loop3 peptide to which it prefers to interact using oriented cyclic peptide library technology. Hence three cyclic libraries were constructed based on the Loop3 structure. Each library held fixed those amino acid residues that are highly conserved or invariant among all of the EGF-like growth factors to provide orientation in binding. Other residues were made 19-fold degenerate in all of the natural amino acids except cysteine to allow for selection of the optimal binding motif by ErbB2. Two of the three synthesized libraries were screened against ErbB2 extracellular domain, and while specific binding to the domain was observed no reproducible selection at degenerate residues was obtained.

The results outlined in this report are disappointing in that no high affinity leads were identified. This is in part due to the fact that the experiments were not fully completed within the funding period because of unforeseen technical difficulties. However, the observation that specific binding of the peptide libraries to ErbB2 may be observed is very encouraging. This strongly suggests that the peptide selection approach using the ErbB2 extracellular domain is a viable means of screening for high affinity peptide ligands. This was the major possible drawback going into these studies. The lack of reproducible selectivity at degenerate residues could result from a number of factors. The most likely of these is that non-conserved residues within the Loop3 region of the peptide do not significantly contribute to the affinity of the peptide for ErbB2. Indeed, some selectivity was observed at residues outside the Loop3 region, but the reproducibility of this result could not be assessed because the experiment was only carried out once. This result also suffered from a low signal-to-noise ratio due to the later cycles of sequencing. But it is quite possible that while the Loop3 region provides the base structure necessary for peptide binding to ErbB2, residues carboxy-terminal to the loop contribute to binding specificity and affinity. This notion will be tested when experiments with library B are repeated and when data with library C becomes available.

In the broader perspective the results from these studies provide a framework for the use of oriented peptide library and cyclic peptide library technology in drug screening for a variety of targets. For example, linear or cyclic peptide libraries directed toward the kinase domain of ErbB2 could be used to uncover potential competitive inhibitors of kinase catalytic activity, although intracellular targets are less attractive because of the difficulty in delivering peptides across the plasma membrane. Particularly attractive targets for breast cancer and other solid tumors are the angiogenic factor receptors, such as receptors for the vascular endothelial growth factors (VEGFs) and the angiopoietins. Moreover, libraries used in drug screens need not be limited to the natural amino acids. Unnatural amino acids such as taurine or norvaline or D-amino acids may also be incorporated into libraries, distinguishing this method from biologically-based techniques such as phage display. In fact the only limitation of peptide library technology in drug screening is that the subunits of the polymer must be able to be determined by Edman degradation. This affords an enormous array of potential compounds that may be synthesized in a very short time frame. Since libraries with as many as eight degenerate residues have been used successfully (17), up to 17 billion compounds may be screened against interesting cancer targets in an afternoon.

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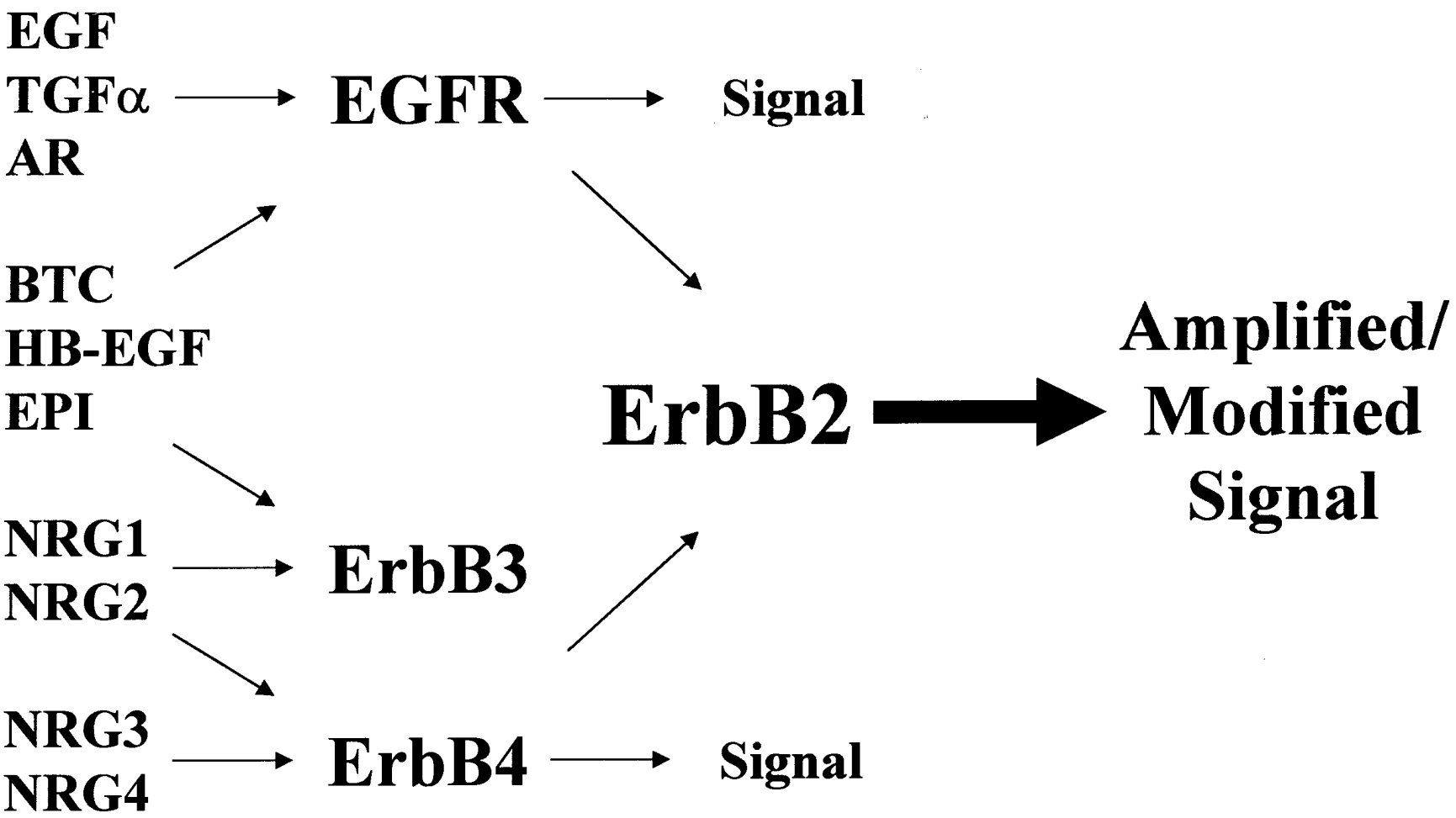


Figure 1. ErbB2 Plays a Prominent Role in Signaling Through the ErbB Receptor Network. Although it has no direct binding ligand ErbB2 plays a significant role in EGF-like growth factor signaling by heterodimerizing with other ErbB family members in response to ligand binding. While homodimers of EGF receptor and ErbB4 mediate biological responses to growth factors, the heterodimerization of ErbB2 with any of the other family member is thought to either augment or modify that response. In the context of a breast tumor, autocrine activation of ErbB2 through ligand stimulation of other ErbB receptors could signifcantly contribute to tumor progression.

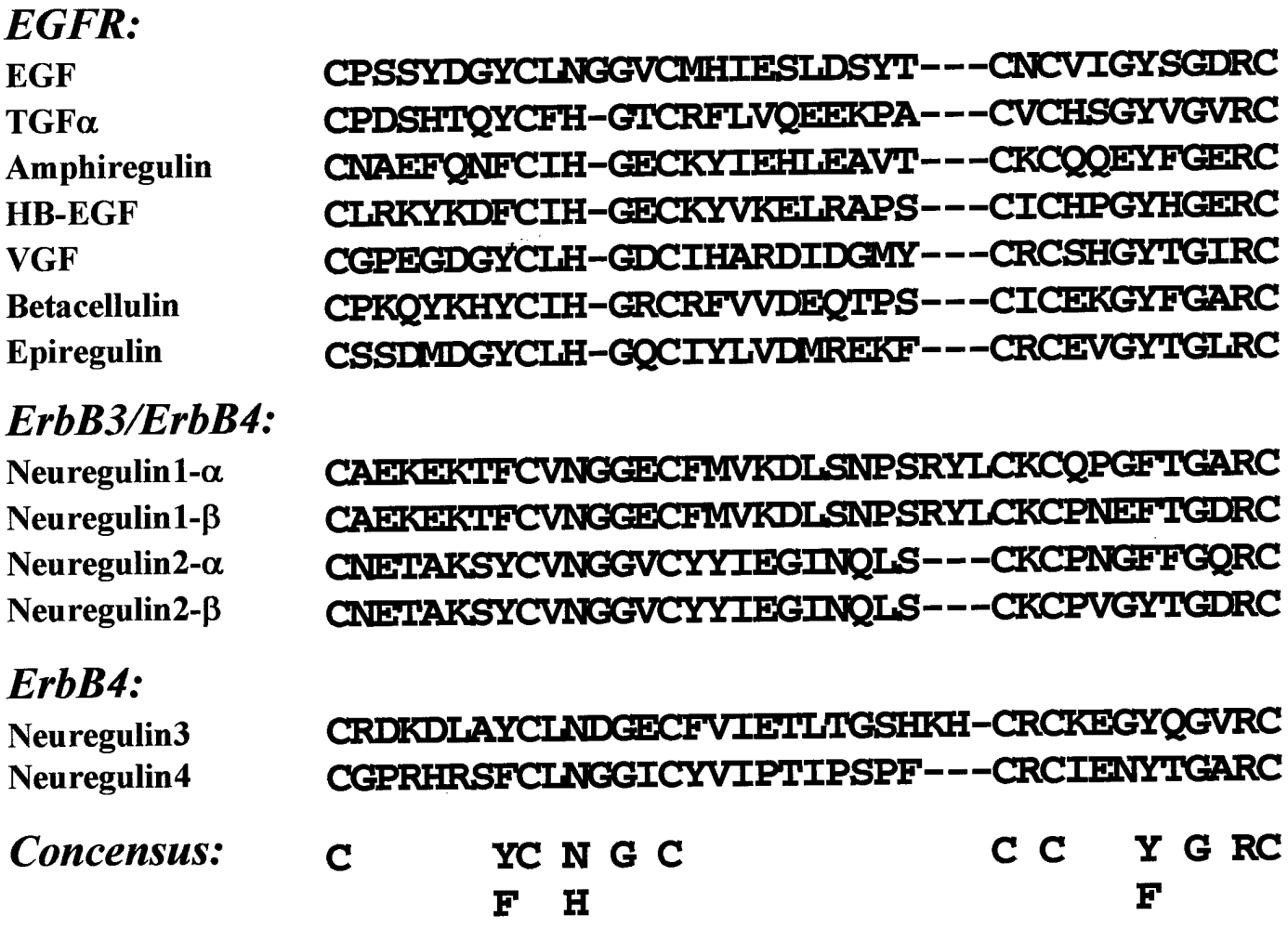
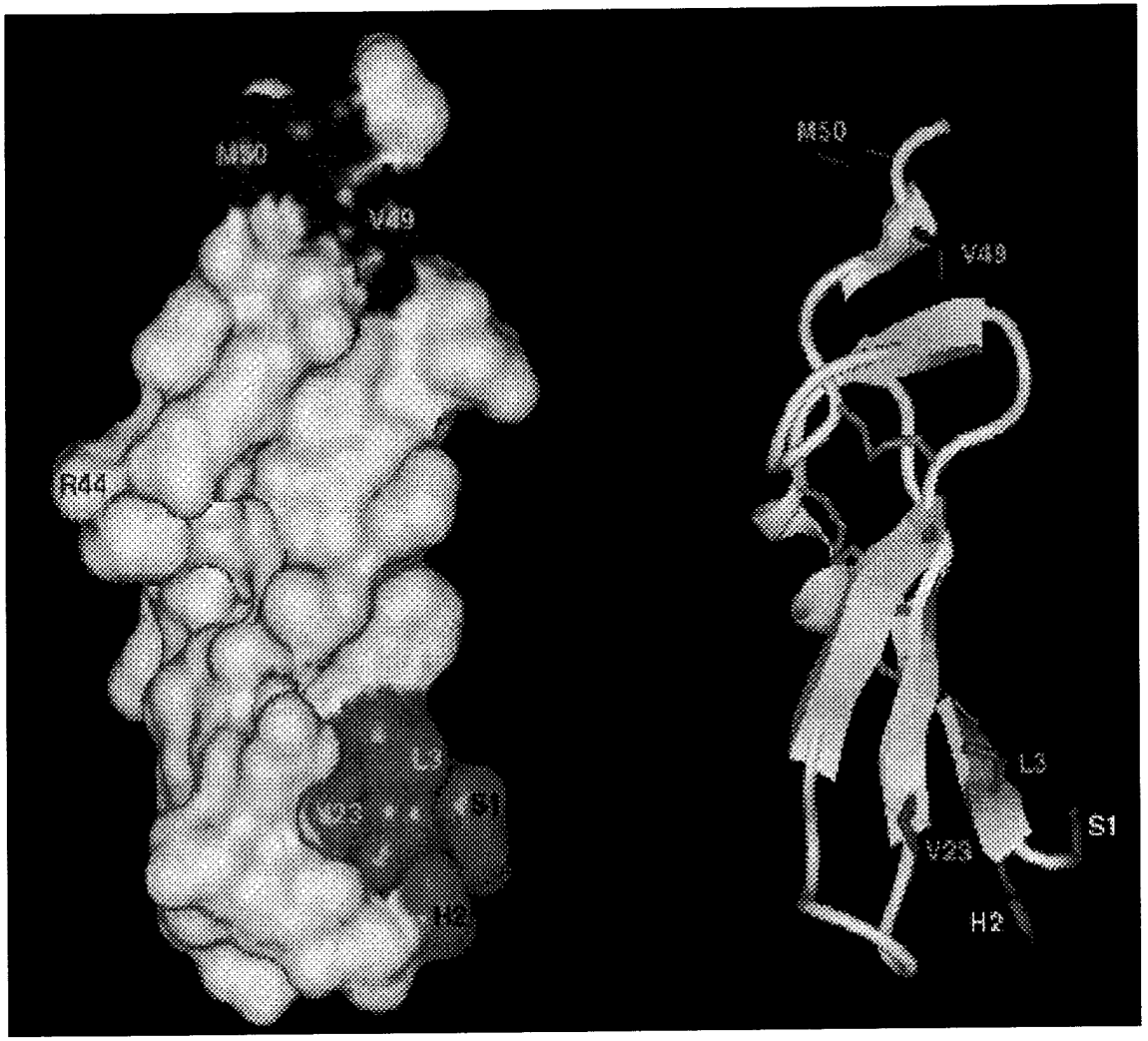


Figure 2. EGF-like Growth Factor Ligands. ErbB receptors are acted on by more than a dozen growth factors that contain epidermal growth factor (EGF)-like domains, the hallmark of which is six chraracteristically-spaced cysteine residues. The first and third cysteines form a disulfide bond, as do the second and fourth. The fifth and sixth cysteine residues form a separate disulfide bond and define Loop3. Many of the known EGF-like growth factors are illustrated here and are grouped according to their primary binding ErbB receptor(s). Notably, no known diffusible ligand acts directly on the ErbB2 receptor.



NRG1β:

SHLVKCAEKE
KTFCVNGGEC
FMVKDLSNPS
RYLCKCPNEF
TGDRCQNYVM

Tzahar et al. *EMBO J.* 16, 4938-4950 (1997).

Figure 3. Bivalence of EGF-like Ligands Drives the ErbB Signaling Network. A widely accepted model for the stimulation of receptor tyrosine kinases by growth factors asserts that ligands are bivalent and can simulataneously make contact with two different receptor molecules. Hence, growth factor bivalency is thought to drive receptor dimerization and kinase activation. For the case of EGF-like ligands, Tzahar et al. have proposed that the amino terminal region of the growth factors is responsible for high affinity, high specificity binding to a primary ErbB receptor target, while the carboxy terminal region (or the Loop3 region along with adjacent sequences) is responsible for lower affinity, less selective binding to a dimerizing ErbB receptor.

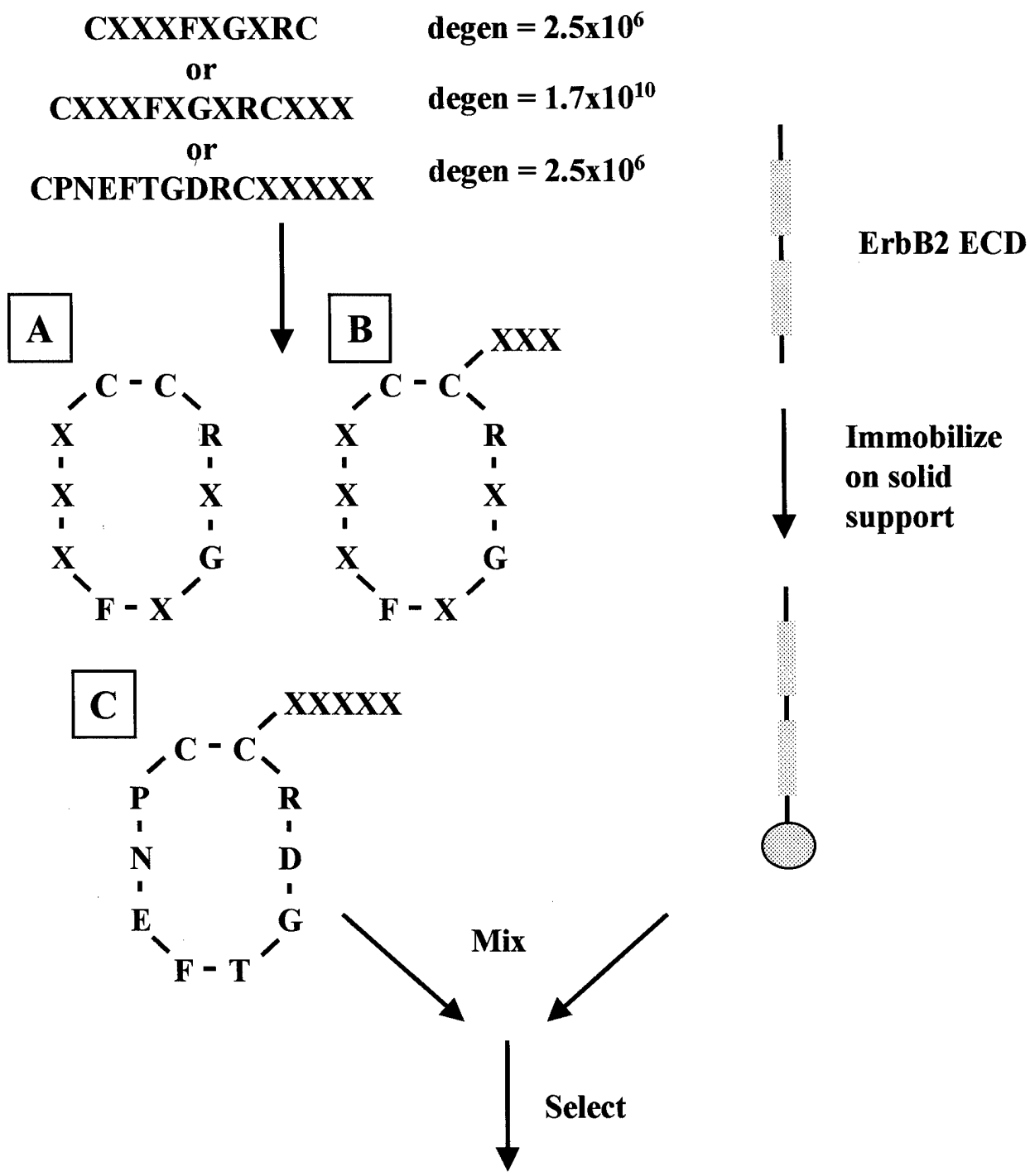


Figure 4. Strategy for the Discovery of High Affinity ErbB2 Antagonoists. A recombinant soluble form of the extracellular domain of ErbB2 is produced in high quantity, purified to homogeneity, and immobilized on beads. Degenerate cyclic peptide libraries based on Loop3 sequences of EGF-like growth factors are incubated with the immobilized receptor, allowing ErbB2 ECD to select those that bind with high affinity. Low affinity and non-specifically bound peptides are washed away, and the high affinity binding peptides are eluted with a low pH buffer. The eluted peptide mixture is sequenced by Edman degradation, and the amino acid content of the eluted material is compared with the original library to determine sequence preferences at each degenerate position. Library [A] is based on Loop3 alone while libraries [B] and [C] add degenerate residues to the carboxy terminus.

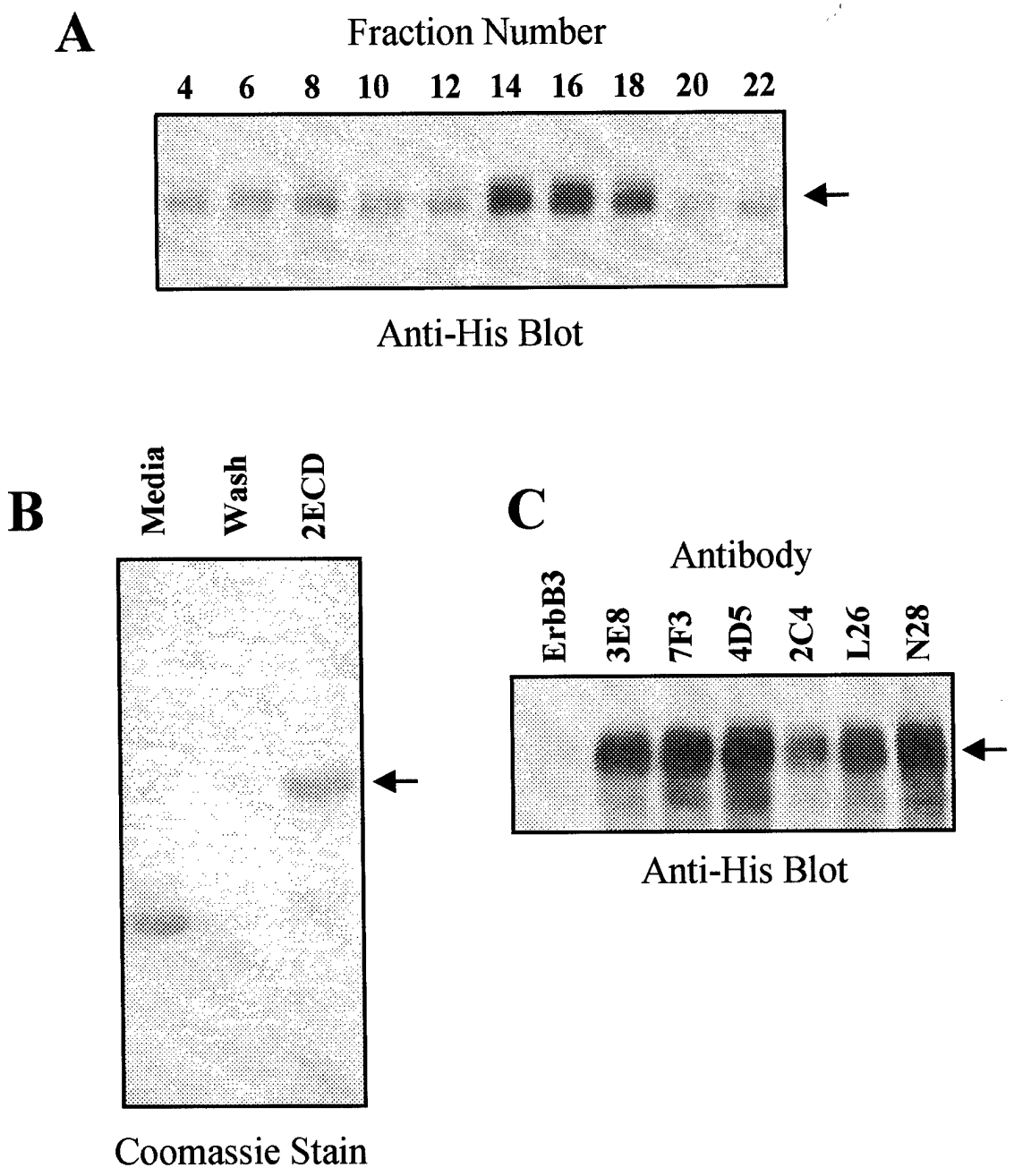


Figure 5. Expression, Purification and Charaterization of ErbB2 ECD. The extracellular domain of ErbB2 was expressed as a secreted amino-terminally 6X-His tagged fusion protein in High Five insect cells. **(A)** The protein was purified from the conditioned media by nickel affinity and eluted with an imidazole gradient. **(B)** While Coomassie staining revealed that the predominant protein in insect cell media was the bovine serum albumin added as a carrier, the predominant protein in ErbB2 ECD was the fusion protein. ErbB2 ECD was estimated to be >90% pure. **(C)** The purified ErbB2 ECD fusion protein was capable of interacting with a battery of conformation-sensitive monoclonal antibodies directed toward human ErbB2, suggesting that the recombinant purified material is functionally intact.

Table 1 – Summary of Cyclic Peptide Library Binding Results

(A) Experiment 1 – Library binding to His-2ECD immobilized on Sepharose. Values are given in pmol sequenced peptide. 1 μmol peptide library was used in each reaction.

	Library A		Library B	
	Phe (cycle 5)	Gly (cycle 7)	Phe (cycle 5)	Gly (cycle 7)
His-2ECD	38	73	12	39
Control	57	98	9	63
2 nmol library	628	771	554	681

(B) Experiment 2 – Library binding to free His-2ECD. Values are given in pmol sequenced peptide. 1 μmol peptide library was used in each reaction.

	Library A		Library B	
	Phe (cycle 5)	Gly (cycle 7)	Phe (cycle 5)	Gly (cycle 7)
His-2ECD	326	441	402	532
Control	79	100	39	47
2 nmol library	888	935	933	1121

(C) Experiment 2 – Selection factors for cycle 6. The mole fraction of each amino acid in the sixth cycle of the eluted peptides was divided by the corresponding mole fraction in the libraries to yield selectivity factors. A factor of 1 indicates no selection for or against that amino acid.

Amino Acid	Library A	Library B
Ala	1.25	0.72
Arg	1.18	1.08
Asn	0.60	0.85
Asp	1.65	0.62
Gln	0.65	0.92
Glu	0.94	1.73
Gly	0.80	1.25
His	1.08	1.47
Ile	1.42	1.01
Leu	0.97	0.81
Lys	0.79	1.62
Met	1.04	0.89
Phe	1.72	2.15
Pro	0.70	1.64
Ser	1.24	0.88
Thr	1.52	0.62
Trp	1.30	0.85
Tyr	0.78	1.16
Val	1.08	1.27